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TITLE: Method of enriching rare cells

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INVENTOR-INFORMATION:

NAME CODE COUNTRY	CITY	STATE	ZIP
Ts'o; Paul O. P. N/A	Ellicott City	MD	N/A
Wang; Zheng-Pin N/A	Towson	MD	N/A
Lesko; Stephen A. N/A	Baltimore	MD	N/A
Nelson; William G. N/A	Towson	MD	N/A
Partin; Alan W. N/A	Baltimore	MD	N/A

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CLAIMS:

What is claimed is:

1. A method for enriching cancer cells in a bodily fluid sample comprising cancer cells and non-rare cells comprising:

(a) obtaining the sample comprising cancer cells and non-rare cells;

(b) subjecting the sample to multiple density gradient separation comprising a first density gradient and a second density gradient, wherein the second density gradient is greater than the first density gradient, and producing a first fluid comprising an increased concentration of cancer cells of a first density, and a second fluid

comprising an increased concentration of cancer cells of a
second density, wherein
the second density is greater than the first density;

wherein subjecting the sample to multiple density gradient
separation includes
producing a plasma layer, a first interface layer, a first
gradient layer, a second
interface layer, a second gradient layer, and a cell pellet;

wherein producing the first fluid includes combining the
first interface layer and
the first gradient layer and forming a first suspension; and

wherein producing the second fluid includes combining the
second interface layer and
the second gradient layer and forming a second suspension;

(c) subjecting said second fluid comprising the second
suspension to a binding agent
that binds non-rare cells;

(d) removing the bound non-rare cells from the second fluid
to provide a second
fluid enriched with the greater density cancer cells, and

(e) preparing a fluid enriched with the cancer cells of the
first density and the
cancer cells of the greater density by combining the cancer
cells of the first
density from the first fluid and the greater density cancer
cells from the second
fluid enriched with the greater density cancer cells.

2.   The method of claim 1, wherein said cancer cells are
alive during the course of
said method.

3.   The method of claim 1, wherein said cancer cells are
epithelial cells.

4.   The method of claim 3, wherein said epithelial cells are
prostate cancer cells.

5.   The method of claim 4, further comprising characterizing
the prostate cells
using at least one prostate-specific marker expressed by the
prostate cells.

6.   The method of claim 5, comprising detecting at least one of a prostate-specific
antigen and a prostate-specific membrane antigen.

7.   The method of claim 4, further comprising characterizing the prostate cells
using a cytokeratin protein marker expressed by the prostate cells.

8.   The method of claim 6, wherein at least one of the prostate-specific antigen and
the prostate-specific membrane antigen is detected using a nucleic acid probe that
specifically binds to the mRNA of said antigen.

9.   The method of claim 8, wherein said probe is selected from the group consisting
of SEQ. ID. Nos. 1, 2, and 6.

10. The method of claim 8, wherein said probe is selected from the group consisting
of SEQ. ID. Nos. 3, 4, 7, and 8.

11. The method of claim 6, wherein at least one of the prostate-specific antigen
and the prostate-specific membrane antigen is detected using an antibody that
specifically binds to said antigen.

12. The method of claim 3, further comprising characterizing ploidy state of the
epithelial cell using at least one centromere specific marker.

13. The method of claim 12, wherein the centromere specific marker comprises a
nucleic acid probe that specifically binds to a complementary sequence of the
centromere DNA.

14. The method of claim 13, wherein said probe comprises SEQ. ID. No. 5.

15. The method of claim 13, wherein said probe comprises SEQ. ID. No. 10.

16. The method of claim 1, wherein said binding agent

comprises an antibody.

17. The method of claim 16, wherein said binding agent comprises at least two
primary antibodies from animals that are capable of binding to different non-rare
cell antigens.

18. The method of claim 16, wherein said binding agent comprises a primary antibody
from an animal that binds to a non-rare cell, and a secondary anti-antibody from
another species than the primary antibody, wherein said secondary anti-antibody
binds to the primary antibody.

19. The method of claim 17, wherein the at least two primary antibodies are capable
of binding to human non-rare cell antigens, and the binding agent further comprises
secondary antibodies capable of binding to the two primary antibodies, wherein the
primary antibodies are from a different species than the secondary antibodies.

20. The method of claim 1, wherein subjecting the sample to density gradient
separation comprises using at least one density gradient medium having a density of
no less than about 1.06 g/ml.

21. A method of detecting cancer cells in a fluid comprising cancer and non-rare
cells, which method comprises providing a fluid enriched with cancer cells by the
method of claim 1, and analyzing said fluid to detect said cancer cells.

22. A method of detecting prostate cancer cells comprising providing a fluid
enriched with prostate cancer cells by the method of claim 4, and analyzing said
fluid to detect the prostate cancer cells.

23. The method of claim 3, further comprising determining the number of chromosomes
in the cancer cells.

24. The method of claim 4, further comprising determining the number of chromosomes
in the prostate cancer cells.

25. The method of claim 23, including determining the presence of aneuploidy in the
cancer cells.

26. The method of claim 3, comprising increasing by at least about 500-fold the
concentration of the cancer cells compared to the concentration of the cancer cells
to the non-rare cells in the fluid sample.

27. The method of claim 1, wherein the cancer cells are human liver cells, hepatoma
cells, or hepatocarcinoma cells.

28. The method of claim 1, wherein said fluid is blood.

29. The method of claim 1 comprising subjecting the second fluid comprising an
increased concentration of cancer cells of a second density to a binding agent that
binds white blood cells and red blood cells, and removing the bound white blood
cells and bound red blood cells from the second fluid to provide a second fluid
enriched with the greater density cancer cells.

30. The method of claim 1, wherein the cells are alive during the course of said
method.

31. The method of claim 1, wherein the non-rare cells comprise blood cells.

32. The method of claim 31, wherein the blood cells comprise leukocytes and red
blood cells.

33. A cancer cell enriched fluid prepared in accordance with the method of claim 1.

34. The method of claim 1, wherein the first density gradient has a density in the
range of about 1.068 g/mL to about 1.077 g/mL, and wherein the second density

gradient has a density in the range of about 1.077 g/mL to about 1.085 g/mL.

35. The method of claim 3, wherein the epithelial cancer cells comprise breast
cancer cells.

36. The method of claim 3, wherein the epithelial cancer cells comprise kidney
cancer cells.

37. The method of claim 1, wherein subjecting the second fluid to the binding agent
includes binding the white blood cells and/or red blood cells to magnetic beads.